PB83-153957

Pesticide Assessment Guidelines, Subdivision L, Hazard Evaluation Nontarget Insects

(U.S.) Environmental Protection Agency
Washington, DC

15 Oct 82

U.S. Department of Commerce
National Technical Information Service
NTIS

PB83-153957

EPA 540/9-82-019
October 15, 1982

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION L

HAZARD EVALUATION: NONTARGET INSECTS

by

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	3. Recipient's Accession No. PB83 153957
4. Title and Subtitle: Subdivision L - Hazard Evaluation: Nontarget Insects		5. Report Date: August, 1982
		6.
7. Author(s): Ecological Effects Branch, Hazard Evaluation Division		8. Performing Organization Rept. No.
9. Performing Organization Name and Address: Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460		10. Project/Task/Work Unit No.
		11. Contract(C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address: Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460		13. Type of Report & Period Covered: Guideline
		14.

15. Supplementary Notes

Guidelines Coordinator: Robert K. Hitch

16. Abstract (Limit: 200 words)

40 CFR, Part 158, § 158.31 specifies the data on toxicity to nontarget insects which are required to support the registration of a pesticide product. Subdivision L provides guidelines for the development of that data required in 40 CFR, Part 158. Each section in Subdivision L specifies the conditions under which specific data are required, provides requirements for reporting and evaluation of data. Also, each section contains suggested protocols, references to protocols, or both.

Subdivision L constitutes one volume of a twelve-part FIFRA guideline series published by the National Technical Information Service.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement

19. Security Class (This Report)	21. No. of Pages: 34
20. Security Class (This Page)	22. Price

Foreword

Subdivision L describes protocols which may be used to perform nontarget insect toxicity testing to support the registration of pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). It is a nonregulatory companion to 40 CFR Part 158, Data Requirements for Registration. Public comment on Subdivision L has been taken in a series of public meetings, the last of which was held in July, 1982. Data requirements established by 40 CFR Part 158 are discussed in Subdivision L so that it can be read as a complete package and so that nontarget insect testing procedures can be explained in their proper context.

Subdivision L

HAZARD EVALUATION: NONTARGET INSECTS

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I. ORGANIZATION AND PHILOSOPHY OF SUBDIVISION L

A. Introduction.

Subdivision L provides guidelines for testing and information on data submission concerning the effects of pesticides on non-target insects. Data developed according to the guidelines in this subdivision, in conjunction with information obtained through other subdivisions of the guidelines, will be used to make the determination, required by FIFRA, as to whether a pesticide will perform its intended function without causing unreasonable adverse effects on the environment.

B. Approach

Proposed rule, 40 CFR Part 158, specifies the kind of data and information that must be submitted to EPA to support the registration of each pesticide under the Federal Insecticide, Fungicide and Rodenticide Act. The Agency intends to promulgate Part 158 as a final rule during 1983. This subdivision provides detailed information relating to the data requirements listed in 40 CFR Part 158, including the conditions under which each data requirement is applicable, the standards for acceptable testing, stated with as much specificity as the current scientific disciplines can provide, and the information that should be included in a test report.

C. Tier System.

To adhere to the guidelines of this subdivision, the applicant must submit data obtained from specified toxicity tests. These tests have been grouped into 3 broad areas: tests for pesticidal effects on pollinators (§§ 141-1, -2, -3, -4, and -5); tests for pesticidal effects on aquatic insects (§§ 142-1, -2, and -3); and tests for pesticidal effects on insect predators and parasites (§§ 143-1, -2, and -3).

Tests for pesticidal effects on pollinators are organized in a hierarchical, or tier type, system. Generally, the decision as to whether to proceed to the second tier, or longer term tests, is based on the potential toxicity demonstrated in the first level tests (tier I), in conjunction with other pertinent information such as use pattern and environmental fate profile. Third level tests (tier III) are designed to provide additional information with respect to adverse results or conditions reported in lower tier studies, and are performed under simulated or actual field conditions. Third level tests will rarely be required, as most questions concerning pesticide hazard will be answered by the first and second level tests.

Guidelines for aquatic insect testing and for insect predator and parasite testing have not been developed to date. The issues raised in establishing such guidelines are discussed in detail in part II of this discussion.

D. Organization of Sections Within Subdivision L.

Within Subdivision L, each section provides the guidelines for a particular test or group of related tests, and contains the following: a provision stating the circumstances under which data are required; a provision stating whether testing must be performed on the manufacturing-use product or end-use product, or both; provisions establishing the test standards that must be complied with in generating the data; and specific reporting requirements. In addition, an applicant must comply with the applicable provisions of CFR 40, Part 158, which establishes general requirements applicable to most pesticides.

1. "When required" paragraphs. Each of the sections of this subdivision establishes data requirements for a particular test and begins with a paragraph entitled "When required." This paragraph establishes the conditions under which data from that test are required to support the registration of a pesticide product.

Virtually every manufacturing-use product is formulated into an end-use product the use of which can pose a risk to non-target insects. Therefore, the first tier studies in Subdivision L (§§ 141-1, -3, 142-1, 143-1, -2, and -3) apply to all manufacturing-use products, regardless of eventual intended use, and to all end-use products intended for outdoor application, subject to any exceptions contained in each section.

2. "Test standards" paragraphs. Specific test standards for a study are set forth in the paragraph entitled "Test standards" in each section. In addition, testing must be performed in accordance with the "Basic standards for testing" contained in § 140-3. The general and specific test standards for acceptable testing identify the factors in the performance of a test that EPA has determined to be necessary to produce reliable and complete data. The test standards cover such aspects of testing methodology as the test substance, test conditions, test species, age of test organisms, and duration of the test.

3. Reporting requirements. Section 140-4, "Reporting and Evaluation of Results," provides the general reporting and evaluation requirements for this subdivision. In addition, each section contains a paragraph entitled "Reporting of data," which sets forth the Agency requirements for the data that must be submitted. In

most cases, the minimum data required to support the registration of a pesticide are described in the first level tests, §§ 141-1(c) and 141-3(c). These data include a determination of the acute contact LD_{50} for honey bees for each active ingredient of the pesticide, and a determination of the residual toxicity of end-use pesticide products to alfalfa pollinators.

Minimum data requirements for aquatic insects and for insect predators and parasites have not been developed to date.

The Agency intends that the need for data submission required by §§ 141-2, -4 and -5 be relatively infrequent. However, the Agency believes that information about these tests should enable applicants to plan their testing programs more effectively.

4. Protocols and references. Subdivision L includes paragraphs at the end of each section that provide examples of acceptable test protocols and/or references that may contain background information useful in developing acceptable protocols. This information is intended only as guidance to the applicant.

II. MAJOR ISSUES

Agency review of the Subdivision L guidelines has identified a number of issues which are discussed in the following paragraphs.

A. Data Requirements for Manufacturing-Use Products.

In the Preamble to the 1978 proposed Guidelines, EPA asked for public comment on the question whether the data requirements of this subdivision should be extended to manufacturing-use products. After serious consideration of this issue, the Agency has concluded that extending the data requirements to such pesticides is appropriate. The Agency was influenced by the views of commenters on this issue who generally favored a data submission requirement which makes the basic manufacturer of an active ingredient responsible for providing most of the environmental fate data.

Therefore, 40 CFR § 158.50, entitled "Formulators' Exemption," requires a registrant of a manufacturing-use product to submit (or cite) any data pertaining to the safety of an active ingredient in its product if the same data are required to support the registration of an end-use product that could legally be produced from the registrant's manufacturing-use products. (An end-use product is a pesticide product bearing label directions for immediate end-use as a pesticide). Section 158.50 also provides that such data must be submitted by an applicant for registration of the end-use product, except that the producer of the end-use product will generally not

have to submit or cite data pertaining to registered products which the end-use producer purchases and uses to formulate the end-use product. This decision reflects the Agency's expectation that manufacturing-use product registrants will be the major source of registration data, and that end-use product formulators will, in most cases, need to supply much less data. This decision is consistent with the provisions of, and Congressional intent behind, sec. 3(c)(2)(D) of FIFRA, which provides that:

> No applicant for registration of a pesticide who proposes to purchase a registered pesticide from another producer in order to formulate such purchased pesticide into an end-use product shall be required to--
>
> (i) submit or cite data pertaining to the safety of such purchased product; or
>
> (ii) offer to pay reasonable compensation otherwise required by [§ 3(c)(1)(D) of FIFRA] for use of any such data.

Implicit in sec. 3(c)(2)(D) is Congress' expectation that it would be the registrant of the manufacturing use product who would provide significant amounts of data pertaining to the safety of its product. (See, e.g., Sen. Rep. No. 334, 95th Cong., 1st Sess., pp. 8-9.)

Moreover, if data requirements were imposed solely on registrants of end-use products, sec. 3(c)(2)(D) might be read to prevent the Agency from obtaining data on the grounds that the data pertain to the safety of a purchased product.

B. Testing for Effects on Terrestrial Insect Predators and Parasites. Testing for adverse effects on insect predators and parasites has been a major topic of discussion and disagreement. Those who oppose the establishment of predator/parasite data requirements cite a number of reasons. The regulatory use of such information has been questioned on the basis that it is unclear how such information can be used in product labeling. Doubt has been expressed as to the predictive value of pesticide effects data on only a few major species, when certain agroecosystems are known to contain hundreds of species of nontarget insects. Finally, some reviewers believe that establishment of predator/parasite data requirements will require an unwarranted expenditure in terms of time and money for development, submission, and evaluation of the data.

Those who favor the establishment of data requirements for insect predators and parasites reason that assessment of this hazard is an inherent part of the Agency's responsibility for overall nontarget organism hazard evaluation, and that such information is essential to support the development of strategies under integrated pest management (IPM) programs. (See also part II.E. of this discussion.)

If the guidelines should address the hazard to predators and parasites, the question then becomes one of procedure; i.e., what is the best way to go about making this hazard assessment? Several approaches have been suggested.

One approach would break down representative predators and parasites according to major agroecosystems. Key species (usually less than six) would be selected from each agroecosystem for laboratory screening tests and, if necessary, for field tests. Data on these representative species could be used to extrapolate potential adverse effects to other nontarget species. This scheme would indeed indeed provide a solid base for pesticide hazard assessment, but the time involved in generating and reviewing the data might be prohibitive. It has also been suggested, as a means of reducing time and cost, that only three to four representative predator/parasite species be selected overall for testing. Such representative species would probably be selected based on their widespread "distribution" and importance in major agricultural crop systems.

Finally, it has been proposed that, instead of imposing data requirements, the Agency might create an "incentive" for "beneficial effects" testing. In other words, applicants who demonstrate that their products have utility within IPM systems could be permitted to incorporate positive label statements regarding the absence of adverse effects of their products on nontarget insects. The Agency's decision whether to permit such claims would be based on review of data voluntarily submitted by the registrant. Implementation of this system would require an amendment of Subdivision H (Labeling Requirements for Pesticides and Devices) to permit appropriate nontarget insect safety claims on product labels.

C. Testing for Effects on Aquatic Insects.

The sections of Subdivision L that deal with aquatic insect testing have been designated as reserved. This action was taken for the following reasons:

1) Subdivision L requirements duplicate those in Subdivision E to a degree. Subdivision E data requirements provide substantial information which could be used for aquatic insect hazard assessment.

2) Methodology for testing for pesticide effects on nontarget aquatic insects is not well developed at this time, especially for testing beyond the acute toxicity level (e.g., testing for chronic or reproductive effects).

D. Scope of Testing.

Inherent in guidelines development is the need to strike a balance between adequate data requirements and increased regulatory burden. With this in mind, the Agency has considered a number of potential data requirements which have not been included in the draft to date. Generally, the reason for their exclusion is a lack of methodology for generating the data. Since a decision to include certain additional requirements would involve development and validation of methods, the decision must be based on the overall importance of the particular data requirements.

Specifically, consideration has been given to requiring the following types of tests: effects of pesticide application (especially forest uses) on soil/litter arthropods; effects of systemic pesticides on pollinators and on insect predators and parasites; effects of mosquito abatedment treatments on pollinators; effects of pesticide applications on pollinators other than those already included in this subdivision (e.g., bumblebees); and pesticide effects on introduced biological control agents (insects).

E. Relation of Subdivision L to Agency IPM Program.

The Environmental Potection Agency's Integrated Pest Management (IPM) unit has expressed interest in the development of those Subdivision L sections which deal with insect predators and parasites. The IPM unit has provided suggestions that could serve as the basis for Subdivision L data requirements. Information identified by the IPM unit as useful includes:

- Pesticide efficacy against target pests in an IPM setting;
- Effect on other test species (direct);
- Effect on biological control agents and other beneficial organisms found in IPM ecosystems;
- Most appropriate use pattern in an IPM program;
- Probability of resistance developing in pests and beneficial species; and
- Effect on total pesticide use in the system.

These ideas related to "system impacts" rather than specific effects on selected test organisms. As certain commenters have pointed out, evaluation of "system impact" involves large-scale, long-term studies. Such evaluation would probably be beyond the limited scope of testing now envisioned for Subdivision L.

Even though it may not be possible to structure Subdivision L to respond to the broad issues outlined by the IPM unit, it may be possible to design the predator/parasite testing scheme so that required data are of value to the IPM unit as well as to the rest

of the Agency. In other words, issues outlined by the IPM unti should be kept in mind while the predator/parasite parts of Subdivision L are being designed.

F. Honey Bee Subacute Feeding Study.

Section 141-4 of Subdivision L has been reserved for a honey bee subacute feeding study. With the exception of field studies, the other bee studies in this subdivision are designed to assess pesticide toxicity to individual bees; the importance of the subacute study is that it is designed to assess effects on the colony as a unit.

A honey bee subacute feeding study is currently being developed and validated under an EPA contract. The methodology suggested here and the issues noted below were generated during a November 1978, meeting of EPA and USDA representatives and bee researchers. Participants in the discussion were given the opportunity to comment on a written summary subsequent to the meeting.

Before data requirements are imposed, there is a need to validate the proposed methodology and to develop clearly defined criteria for requiring that the study be conducted.

1. Purpose. The purpose of the honey bee subacute feeding study is to study the effects of low levels of pesticides on honey bee colonies. The study is intended to identify those pesticides which may cause adverse reproductive, behaviorial, or other subacute effects, and which can be brought back to the hive because the foragers exposed to the pesticide are not killed outright in the field. The adverse potential of such a pesticide would not show up in an acute toxicity test, nor is it likely that the fairly short term residual toxicity test would reveal this potential.

2. General approach. The test involves exposure of intact bee colonies to low levels of pesticide through feeding of the pesticide in pollen and in sugar candy or syrup. Through caging or location, colonies are restricted to feeding only on the treated food provided. At the same time, control colonies are maintained under the same conditions, but without exposure to the test pesticide. Periodic checks on amounts of eggs, sealed brood, and adult population size, as well as observations of mortality and physiological or behavioral abnormalities, allow the researcher to determine whether the low level of pesticide is adversely affecting the colony.

3. When required. The Agency believes that requiring the test for every pesticide to which bees might be exposed would not be feasible in terms of the time and money needed for data development and review. Therefore, the Agency intends to restrict testing to

pesticides having characteristics which would indicate a likelihood of the pesticide being transported back to the hive. The honey bee subacute feeding study would be required to support the registration of each end-use product intended for outdoor application if the following three criteria apply:

- Honey bee acute contact LD_{50} testing shows that the LD_{50} of any active ingredient in the product is less than 11 micrograms/bee;

- Residue analysis of foliage performed in accordance with § 132-2 of Subdivision K shows extended presence of dislodgeable residues on plant surfaces; and

- The proposed use involves application to crops that are known to be attractive to bees.

Data from the study would also be required to support the registration of each manufacturing-use product which can be legally and physically used to produce such an end-use product.

4. Length of study. There is no consensus as to the appropriate length of the study. Suggested time periods for the study have ranged from 42 days to four months. (Forty-two days is the approximate time needed for two complete brood cycles.)

Should the researcher choose to use "small" colonies in styrofoam minihives for the test, and provided that monitoring of colony conditions is done in careful detail, 42 days is probably sufficient for detection of even minor adverse effects due to the test pesticide. The suggestion that four months might be necessary is based on the use of full sized colonies and standard equipment.

5. Dosage range. One point that bee research experts agreed upon is that even the highest dosage should not eliminate any of the test colonies during the test period. Beyond that, the following suggestions were made as bases for determining the range of dosage to be tested:

- The dosages and rates of dosing should simulate field exposure;

- Dosages should be related to exposures corresponding to recommended application rates, but should not necessarily equal field exposure; and

- Dosage range can be roughly determined from acute toxicity data.

Simulation of "field exposure" will not be appropriate for all pesticides, since in some instances it would lead to outright

destruction of test colonies. In the case of a known toxic pesticide, even the highest test rate might have to represent some fraction of the exposure expected from a recommended application of the pesticide.

However, test rates should be related somehow to exposures expected from standard applications. In other words, testing might establish the lowest level at which a pesticide adversely affects the colony. The researcher must then determine whether this level of exposure will be attained or exceeded under normal conditions of use.

Regarding suggestion (3)(above), manipulation of acute toxicity data might be a valid method for determination of the dosage range. If this is the case, then the written protocol must provide details and instructions as to how this manipulation is conducted.

A final suggestion was that testing should be conducted using the maximum non-lethal dosage (LC_0) rather than a range of dosages. This would greatly decrease the number of colonies needed for the test.

6. Administration of toxicant. There is agreement that honey bee colonies will be exposed to the test pesticide through treated food substances, with the treated pollen and sugar candy/syrup as the sole food source. However, there is some question as to when the exposure to treated food should begin. Day 1 (day of queen release or package installation) has been suggested, the rationale being that this would be the time of greatest uniformity among the test "colonies." Following this line of reasoning, allowance for any "waiting period" prior to toxicant administration would result in loss of the original uniformity among test units, due to the differences in rate of development among the colonies.

Those who favor some waiting period prior to toxicant administration cite several reasons for this. First, they feel that uniformity among package bee units should not be assumed, due to normal variation among packages and among queens. A waiting period, with the possible establishment of extra colonies beyond the number needed for testing, would allow the researcher to select developed colonies of approximately equal strength. The rationale is that this would provide for more uniformity among test units prior to the actual testing. Also, given the possibility that some of the packages might fail to develop normally, the waiting period would allow the researcher to ascertain this and to replace those failed units with the "extras" prior to testing. In other words, pretest uniformity would be better assured by using this method than by administration of the toxicant on day one. Another reason cited is that the bee package is not a true colony until it has had some time to develop. Thus, exposure to the toxicant from day 1 would not provide a valid test of pesticide effect on a colony.

Those who favor a waiting period to allow for colony development suggested time periods ranging from 10-30 days.

7. Feeding of the colonies. On the subject of feeding the colonies, two major areas of concern have been identified: determination of the appropriate food substance to be used, and development of a system for replacement of food during the test.

For the honey bee colony to develop normally, it must have a supply of protein and a supply of sugar. For the purposes of this test, there are three different protein sources to choose from, and two ways to provide sugar to the colony.

Protein may be provided through feeding of natural pollen, pollen plus supplement, or pollen substitute. There are arguments for and against the use of each. For example, a reliable source of clean natural pollen may be very difficult to find, and the price might be prohibitive. On the other hand, some scientists question whether an adequate pollen substitute has been developed to date, which would allow for normal colony development under the stress of the test conditions. This is another point which must be established through validation of the methodology.

It has been suggested that the choice of using either sugar candy or sugar syrup be left up to the researcher. Appropriate selection would depend to a great degree upon the pesticide being tested. For example, some pesticides tend to settle to the bottom of a sugar syrup, which would result in uneven dosage. In such a case, candy would be the appropriate sugar source.

Food replacement during the test also presents a number of problems which can only be worked out through actual testing. For example, some researchers feel that fresh food should be supplied on a regular basis, with uneaten food being removed at the same time. This would mean replenishment of a fresh supply of toxicant on a regular basis. Others feel that fresh food should be supplied only as needed, allowing complete consumption of the previous food supply. The major point of contention here centers around breakdown of the toxicant over time. Food replenishment on a regular basis might eliminate toxicant breakdown as a factor in the study. Food replacement on an "as needed" basis allows for the toxicant degradation that, as advocates of this method point out, occurs under normal field conditions.

Location of food in the hive has presented no problems, the only suggestion being that food be located on the top bars of the brood combs.

8. Size and number of colonies. There are two major schools of thought on the matter of appropriate colony size for this test. One group advocates the use of standard hives; the other advocates

the use of "mini-hives" which may be of wood or styrofoam. Arguments in support of the use of standard hives are that:

- Use of standard size equipment and colonies represents the real world situation; no extrapolation of data is necessary; and

- Determination of effects on queen (e.g., reproductive capability) can be made easily within the original hive.

Arguments for use of mini-hives are that they are less expensive and require less physical work; and use of small colonies under controlled conditions allows for a clearer expression of adverse effects in a shorter time period.

The first step in resolution of this problem will be to conduct the study using both methods. This will provide needed information on reliability of data obtained, on time and manpower requirements, and on expense. Based on such information, one of the methods may be recommended or required, or the choice of method may be at the option of the applicant. In any case, the number of colonies per treatment level and the number of controls must be reported, and each number must be no less than five.

9. Cages and testing facilities. Appropriate testing facilities might be developed indoors, outdoors, under glass, or under plastic. Whatever the choice, the following conditions must be met:

- Under conditions of the test, it must be shown that control colonies can survive and reproduce normally; and

- Colonies must be maintained under controlled conditions, and provided with pollen and candy/syrup as the sole food source.

The major problem which must be addressed is the potential difficulty of maintaining healthy colonies while isolating those colonies, in some way, from any natural food source. Natural isolation (i.e., with no type of confinement) is an impossibility in many areas of the U.S. Caging or other methods of confinement also present problems, in that the natural behavior of the bees is severely disrupted. Also, if cages are to be used, the question arises as to whether the test hives must be caged individually, or whether all the hives in one treatment can be caged together. It has been suggested that individual caging of hives presents an excessive and unnecessary expense; others believe it to be necessary in order to obtain valid results. This is another point to be worked out through actual testing.

10. Observations. The following measurements must be taken at regular intervals (as yet undetermined): area of eggs, open brood, and sealed brood. Other parameters suggested for evaluation include gross colony weight, estimated adult population, and amount of honey storage. Suggested nonquantitative observations include: presence or absence of disease; discoloration, desiccation, or other abnormalities of eggs and larvae; and morphological or behavioral abnormalities in adults. It has been suggested that dead bee traps might be useful in facilitating observations of abnormalities in brood and adults.

Suggestions on frequency of observations and on timing of first observations have been quite varied. Based on suggestions, measurements as cited above should be taken either once per week, once every two weeks, or only on days 30 and 60 after queen release. It can be assumed that the nonquantitative observations discussed above which involve disruption of the brood nest should be made on the days when measurements are taken.

Use of the dead bee trap allows daily observation without disruption of the colony. With regard to timing of the first observations which involve inspection of the brood nest, it is agreed that some time should be allowed after queen release for the colony to become established. Suggested periods range from two to four days to two weeks. Also, this adjustment period will be correlated with whatever "waiting period" (prior to toxicant administration) has been decided upon.

One final point concerning observations is evaluation of effects on the queen bee. It has been suggested that measuring sealed brood is an adequate measure of effect on the queen's reproductive ability. This might suffice in a test run with standard size hives. If minihives are used, this evaluation will involve placement of the queen in a healthy standard colony at the end of the test period.

11. Pesticide analysis. It has been suggested that pesticide analysis be performed on the treated and control food mixes (as a minimum), and possibly on wax, honey, and dead bees. Appropriate timing and frequency of analysis may depend on a number of factors.

12. Disease control. Disease control in the test colonies can be looked at from two points of view. On the one hand, it may be desirable to make preventative treatments to control the common bee diseases which might interfere with evaluation of pesticide effects. On the other hand, it has been suggested that no such treatments be made, and that disease levels simply be monitored.

13. Minor issues. The following are brief discussions of several minor issues which have not been resolved:

(a) Reuse of equipment. To avoid contamination, it has been suggested that all hive equipment be destroyed after a test. A question has been raised as to whether this is necessary, or whether some method of decontamination could be employed to allow reuse of the hive equipment. The answer to this question could be a major factor in the decision whether to use a mini-hive for testing. Destruction and replacement of mini-hive equipment would not be unreasonably expensive, while regular replacement of standard hive equipment prior to each test might not be feasible from a cost viewpoint.

(b) Water supply. When circumstances require that water be supplied to bee colonies, this is usually done by providing water near the hive entrance or inside the hive. Such provision has been suggested as appropriate for this test. However, another suggestion is that the water supply should be located outside the hive and off the ground, forcing the bees to fly. This would allow for determination of pesticide effect on flight activity, as well as providing the bees with some opportunity to forage outside the hive.

(c) Selection of bee strain. Several suggestions have been offered regarding the selection of bee strains for testing:

- Use Italian worker bees (yellow) and a Midnite queen (black). This would make it easier to differentiate between old and new bees.

- Select reasonably gentle bee stock, since the brood nest must be examined at rather frequent intervals.

- Use one specific strain for all toxicant studies, to allow for standardization of test results.

SUBDIVISION L -- HAZARD EVALUATION: NONTARGET INSECTS

Series 140: SCOPE AND GENERAL REQUIREMENTS

§ 140-1 General information.

(a) Scope. This subdivision addresses the potential adverse effects of pesticides on three categories of nontarget insects: pollinators; aquatic insects; and predators and parasites. Sections 141-1 through 143-3 of this subdivision provide detailed information relating to the data on toxicity to terrestrial and aquatic nontarget insects which are required by 40 CFR § 158.155 to support the registration of a pesticide product. Each section specifies the conditions under which specific data are required. In addition, each section contains standards for acceptable testing (test standards) and a discussion on reporting and evaluation of data. Finally, many of these sections contain suggested protocols, references to protocols, or both.

(b) Application of requirements. (1) "When required" and "test substance" requirements. The registration applicant should be careful to distinguish between the "when required" and the "test substance" paragraphs of each section of this subdivision:

(i) The "when required" paragraphs pertain to the circumstances under which data shall be required by 40 CFR § 158.155, and specify the categories of products for which data must be generated to support registration applications. The test data are ordinarily required to support the registration of each end-use product with the prescribed use pattern and each manufacturing-use product used to make such an end-use product.

(ii) The "test substance" paragraphs refer to kinds of testing required to produce acceptable data, and state the kind of pesticide material that must be used in each test. The test substance for studies in this subdivision may be the technical chemical, a typical end-use product, or an end-use product representative of each different type of formulation that would be used where pollinating insects might be affected. Generally, each of these test substances is prepared by the basic manufacturer of a pesticide chemical.

(2) Testing to meet requirements. Since studies required by 40 CFR § 158.155 would ordinarily be conducted by the basic manufacturer, pesticide formulators would not often be expected to conduct such tests themselves to develop data to support their individual products. They may do so if they wish, but they may also merely rely on the data already developed by the basic pesticide manufacturer.

(c) Tier system of test requirements. (1) Hazard evaluation for pollinators will be based on a hierarchical (tier) testing system. Tests fall into a range from the simplest and least expensive to the more complex and costly. Requirements for testing at the more complex levels will depend on the results from the more basic tests.

(2) The Agency retains the discretion to require additional studies when data required by other sections or subdivisions of the guidelines or derived from other sources (e.g., chemistry or reproductive effects testing) indicate that the pesticide has characteristics, other than acute toxicity, which may pose a hazard to nontarget insects.

(d) Relation of CFR 40, Part 158 to Subdivision L. The registration applicant is referred to CFR 40, Part 158 for purpose of the guidelines, definitions of widely-used terms, and Agency policy on flexibility in relation to deviation from test standards and acceptable protocols.

(e) Waivers. EPA will consider and may grant waivers of data requirements on a case-by-case basis. Every waiver request shall be in writing and must indicate clearly each product and data requirement for which a waiver is requested. A written rationale shall accompany the waiver request. The waiver request shall be submitted to to the Product Manager in the Registration Division who is responsible for the registration of the product to which the request pertains.

§ 140-2 Definitions.

Terms used in this subdivision shall have the meanings set forth in FIFRA, in § 162.3 of the FIFRA sec. 3 regulations, and in §§ 60-2 of Subdivision D and 70-2 of Subdivision E. In addition, for the purposes of this subdivision:

(a) The term "insect," as used in this subdivision, includes the members of the class Insecta (beetles, flies, bees, etc.), and members of the class Arachnida, such as spiders and mites.

(b) The term "nontarget insects" means those insects that are not intended to be controlled, injured, killed, or detrimentally affected in any way by the pesticide for which registration is sought.

(c) The term "contact LD50" means the amount of toxicant per unit weight (expressed as micrograms of toxicant per gram of insect body weight), or per insect, that will kill 50 percent of the test insects exposed to direct application of the toxicant.

(d) The term "typical end-use product" means a pesticide product that is representative of a major formulation category (e.g., emulsifiable concentrate, granular product, wettable powder) and contains the active ingredient of the registration applicant's product.

§ 140-3 Basic standards for testing.

The standards contained in this section apply to all studies in this subdivision unless another section of this subdivision contains a specific standard on the same subject. In such a case, the specific standard shall apply.

(a) Test methods. (1) Toxicity tests should be conducted according to uniform methods, whenever possible, to maximize the number of reliable comparisons that can be made concerning relative toxicity and relative sensitivity.

(2) Tests should include concurrent control groups to determine if any observed effects have developed or occurred independent of the test substance.

(3) Field tests presenting data in terms of reduction (or nonreduction) of numbers of nontarget insects should be designed to include pre-application counts as well as post-application counts.

(b) Test substance. (1) Sections 141-1 through 143-3 of this subdivision specify whether the data submitted in support of an application for registration should be derived from tests conducted with the technical grade of the active ingredient or the end-use product, or both.

(2) The technical grade of the active ingredient is commonly the same substance as the manufacturing-use product for which registration is sought or which is used to produce the end-use pesticide product for which registration is sought. In this case, where these guidelines require testing of the technical grade of the active ingredient, a sample of the manufacturing-use product shall be tested.

(3) Some sections require testing with a typical end-use product.

(4) In addition to or in lieu of testing otherwise discussed in this subdivision, the Agency may require testing to be conducted with:

(i) An analytically pure grade of an active ingredient;

(ii) The technical grade of an active ingredient;

(iii) The inert ingredient(s) of a pesticide formulation;

(iv) A contaminant or impurity of an active or inert ingredient;

(v) A plant or animal metabolite or degradation product of an active or inert ingredient;

(vi) The pesticide formulation;

(vii) Any additional substance which could act as a synergist to the product for which registration is sought; or

(viii) Any combination of substances in paragraphs (b)(4)(i) through (vii) of this section.

(5) The test substance shall be within the limits, if any, certified in accordance with the requirements of § 62-2 of Subdivision D - Chemistry Requirements: Product Chemistry. The composition of the test substance should be determined, including the name and quantities of known contaminants and impurities, so far as is technically feasible. The determination should also include quantities of unknown materials, if any, so that 100 percent of the test sample is accounted for.

(6) The lot and sample numbers of the test substance should be determined and recorded.

(7) The test substance should be stored under conditions that maintain its stability.

(8) If a carrier or vehicle is used to dissolve or dilute the test substance, it should be chosen to possess as many of the following characteristics as possible:

(i) It should not interfere with absorption, distribution, metabolism, or retention of the test substance;

(ii) It should not alter the chemical properties of the test substance and not enhance, reduce, or alter the toxic characteristics of the test substance;

(iii) It should not affect the food and water consumption of the test insects; and

(iv) At the levels used in the study, it should not produce physiological effects or have local or systemic toxicity in insects. In addition, such a carrier or vehicle should, if possible, closely

resemble, as to solvent polarity, the carrier or vehicle to be used under expected conditions of use.

(c) Care and selection of test insects. (1) All data submitted in support of an application for registration should be derived from tests conducted in accordance with good laboratory or field practices for handling and caring for test insects. Only healthy insects should be used, and they should be kept in conditions conforming to proper cultural practices.

(2) Insects selected for testing should be common or representative species currently established in the United States; they may be laboratory-reared or field-collected.

(3) Insects should be randomly assigned to test groups to minimize bias and assure comparability of pertinent variables.

(4) The number of insects tested per concentration and the number of concentrations or dosage levels evaluated should be sufficient to yield statistically sound data.

(5) The insects in each test should, as nearly as practicable, be of uniform size, age, and sex.

(6) In no circumstances shall threatened or endangered species be used as test organisms.

(d) Observations. Observations should be made as frequently as necessary to record visible signs of toxicity and abnormal behavior.

§ 140-4 Reporting and evaluation of results.

Each test report submitted under this subdivision should satisfy the recommendations for reporting and evaluation of data in this section, unless a specific section elsewhere in this subdivision directs otherwise. The test report should include all information necessary to provide a complete and accurate description of test procedures and evaluation of test results. The test report should include a summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary should be sufficiently detailed to permit the reader to understand independently the conclusions of the author. Data should be reported using the metric system.

The test report should include the following information:

(a) Test method. (1) Statement of test method used and a full description of the experimental design and procedures;

(2) The length and actual dates of the study;

(3) The name and address of the laboratory performing the test, and the location where the test was performed; and

(4) The name(s) of the principal investigator(s).

(b) Test substance. (1) Identification of the test substance, including chemical name, and

(2) Manufacturer and lot and sample numbers of the test substance.

(c) Test insects. (1) Identification of test insects (scientific names);

(2) Rationale for selection of species, if species used is other than that specified or preferred in this subdivision;

(3) Age, sex, developmental stage, size, and weights of test insects, as applicable;

(4) Source of supply of the insects;

(5) Strain or colony designation of the test insects, if appropriate;

(6) Method used in assigning test insects to test and control groups; and

(7) Description of any pretest conditioning, including diet.

(d) Dosing or treatment. (1) Description of method, route, and frequency of administration of test material;

(2) Rationale for selection of method, route, or frequency of administration, if it is different from that recommended in this subdivision;

(3) Total volume of material administered (test substance plus carrier);

(4) Identification of any diluents or other materials used in administering the test substance;

(5) Concentrations of test substance(s) administered (i.e., micrograms of test substance per milligram of body weight of the insect [or micrograms of test substance per test insect] or parts per million of the test substance in substrate, medium, or water) or application rates of test substance(s) expressed as pounds of active ingredient per acre and kilograms of active ingredient per hectare;

(6) Description of the dosing or treatment of control insects; and

(7) If the test substance is administered in the diet or water, the assay method used to determine the concentrations of the test substance.

(e) Observations. (1) Frequency, duration, and method of observation;

(2) Detailed description of the nature, incidence, time of occurrence, severity, and duration of all observed toxic effects, including death and any other abnormal or unusual signs and symptoms.

(f) Environmental conditions. (1) Terrestrial species. A description of the rearing conditions during and prior to the test, including:

(i) Ambient temperature and humidity;

(ii) Photoperiod and lighting;

(iii) A description of the diet, including identification and/or composition and sources of diet; and

(iv) Source of water.

(g) Data analysis. (1) Tabulation of the response data at each treatment level;

(2) Calcuation of the LD_{50}, and the 95 percent confidence intervals when sufficient doses and test organisms are used to establish a dose-response line;

(3) Methods of calculation;

(4) No observed effect level; and

(5) Statistical methods used for analysis of data.

(h) References. Complete reference to any published literature and copy of any unpublished literature used in developing the test protocol, performing the testing, making and interpreting observations, or compiling and evaluating the results should be submitted.

§ 140-5 Special test requirements.

In addition to the data requirements outlined in §§ 141-1 through 143-3 of this subdivision, data derived from other tests may, under certain circumstances, be required by the Agency for making judgments regarding safety to nontarget insects. Such data will be required where special problems are involved, and methods may usually be derived from tests already described or cited in other subdivisions of these guidelines. Such data requests may be related to a proposed pattern of use, a toxicological mode of action, or a unique chemical property.

Series 141: NONTARGET INSECT TESTING -- POLLINATORS

§ 141-1 Honey bee acute contact LD50.

(a) When required. (1) End-use products. Data on the acute contact toxicity (LD50) are required by 40 CFR § 158.155 to support the registration of each end-use product intended for outdoor application, when the proposed use pattern indicates that honey bees may be exposed to the pesticide.

(2) Manufacturing-use products. Data on the acute contact toxicity (LD50) are required by 40 CFR § 158.155 to support the registration of each manufacturing-use product which can legally and physically be used to produce an end-use product subject to the data requirement in paragraph (a)(1) of this section.

(3) See 40 CFR § 158.50, "Formulators' Exemption," to determine whether these data must be submitted. Section II-A of this Subdivision provides an additional discussion on this subject.

(b) Test standards. In addition to satisfying the general test standards contained in § 140-3, a honey bee acute contact LD50 study should meet the following standards:

(1) Substance to be tested. The technical grade of each active ingredient in the product shall be tested;

(2) Species. Testing shall be performed on the honey bee, Apis mellifera L.;

(3) Age. Test insects should be worker bees of uniform age.

(c) Reporting of data. Information to be reported should meet the general reporting guidelines of § 140-4, except that in cases where the LD50 will be in excess of 25 micrograms/bee, no

calculated median response level or 95 percent confidence limits are required.

(d) Acceptable protocol. An acceptable protocol may be found in the following reference:

Atkins, E.L., Jr., L.D. Anderson, and T.O. Tuft. 1954. Equipment and technique used in laboratory evaluation of pesticide dusts in toxicological studies with honey bees. J. Econ. Entomol. 47(6):965-969.

(e) Reference. Additional information concerning modification of the original methods may be found in the following reference:

Atkins, E.L., E.A. Greywood, and R.L. Macdonald. 1975. Toxicity of pesticides and other agricultural chemicals to honey bees: Laboratory studies. Univ. of Calif. Div. of Agric. Sci., Leaflet 2287. 38 pp.

§ 141-2 Honey Bee - Toxicity of Residues on Foliage.

(a) When required. (1) End-use products. Data on residual toxicity to bees are required by 40 CFR § 158.155 to support the registration of each end-use product intended for outdoor application:

(i) When the proposed use pattern indicates that honey bees may be exposed to the pesticide; and

(ii) When the formulation contains one or more active ingredients having an acute contact LD50 of less than 11 micrograms/bee.

(2) Manufacturing-use products. Data on residual toxicity to bees are required by 40 CFR § 158.155 to support the registration of each manufacturing-use product which can legally and physically be used to produce a formulated product subject to the data requirement in paragraph (a)(1) of this section.

(3) See 40 CFR § 158.50, "Formulators' Exemption," to determine whether these data must be submitted. Section II-A of this subdivision provides an additional discussion on this subject.

(b) Test standards. In addition to satisfying the general test standards contained in § 140-3, this study should meet the following standards:

(1) Substance to be tested. The test substance shall be a typical end-use product.

(i) If an applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product, the test substance shall be a product representative of a major formulation category which includes that end-use product. If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study should be performed with a typical end-use product for each such category.

(2) Species. Testing shall be performed on the honey bee, Apis mellifera L.

(3) Age. Test insects should be worker bees of uniform age.

(4) Experimental design. A randomized block design should be used in assigning field plots.

(5) Residue analysis. Residue analysis of foliage should be conducted on samples used in the testing, in accordance with § 132-2 of Subdivision K.

(6) Application rates. The test substance should be applied at the proposed label rate(s).

(c) Reporting of data. In addition to the information specified in § 140-4 of this subdivision, the test report should contain the following information, to be obtained at the time of pesticide application and for the duration of the study:

(1) Weather conditions during and after application;

(2) Amount and type of precipitation during residue weathering period; and

(3) Temperature and humidity data for the period following application.

(d) Acceptable protocol. The following is an example of an acceptable protocol:

Introduction

The purpose of this test is to measure the toxicity of field-weathered pesticide residues to honey bees. The formulation is applied at proposed label rates and the residues are allowed to weather under natural conditions. At specific periods of time following application,

treated foliage is collected and bees are confined on the foliage. This allows for determination of the duration of residual toxicity of a pesticide to honey bees. To reduce the variability inherent in any type of field testing, the following suggestions are made:

(1) Weather conditions should be carefully monitored during the testing (precipitation and temperature are two extremely important factors in the breakdown of pesticide residues);

(2) Tests should be replicated over time to reduce variability due to weather conditions;

(3) Test bees should be obtained from several different colonies, as bees from certain colonies may exhibit increased tolerance to some pesticides.

Materials and Methods

Test crop. The preferred test crop is alfalfa; an extensive amount of residual toxicity tseting has been conducted using this crop. Another crop may be substituted, however, in situations where alfalfa may not be feasible or appropriate.

Plots. Small plots (suggest 0.01 acre) should be designated in alfalfa which has been grown according to standard agronomic practices. As noted above, a randomized block design should be employed for the crop.

Pesticide application. The formulated product should be applied at the maximum proposed rate. Lower rates may be tested at the discretion of the registrant.

Test residues. Residues should be allowed to weather in the field for a specific time prior to collection of foliage samples for testing. For purposes of comparison, test samples could be collected 3, 8, and 24 hours after application. If mortality of bees exposed to 24-hour-old residues is greater than 25 percent, sampling at 24-hour intervals should continue until mortality of bees exposed to the treated foliage is not significantly greater than control mortality. Foliage samples should be chopped and mixed prior to introduction of bees. Approximately 500 cc of treated foliage should be placed in each cage.

Cages. Simple cages are constructed with the tops and bottoms of 150x15 mm plastic petri plates. Wire screen is cut into a strip 18x2 inches (46x5 cm) and the ends stapled to form a cylinder. Petri plates serve as top and bottom of the cage.

Feeding. Bees should be fed during testing by providing cotton squares (2x2 in or 5x5 cm) soaked with 50 percent sugar syrup and placed under the treated foliage.

Treatment and testing of bees. Worker bees should be collected from the frames of established colonies. Bees may be anesthetized with CO_2, if necessary, prior to their introduction into test cages.

Fifty to 100 bees should be introduced into each cage. Bees should be caged with the treated foliage and the cages held at 75-78°F (24-25.6°C) during the test period.

Mortality should be determined after 24 hours of exposure to the treated foliage.

A minimum test shall consist of at least three cages of bees per replicate, and each treatment, including controls, shall be replicated at least three times.

(e) References. The suggested protocol was developed from the following references, which may provide additional information:

(1) Johansen, C., C. Kious, G. Schultz, R. Gupta, R. Madsen, and W. Robinson. 1977. Bee research investigations, 1977. Dept. of Entomol., Wash. St. Univ. Unpubl. 22 pp.

(2) Lagier, R.F., C.A. Johansen, M.G. Kleinschmidt, L.I. Butler, L. M. McDonough, and S.D. Jackson. 1974. Adjuvants decrease insecticide hazard to honey bees. Coll. of Agric. Res. Center, Wash. St. Univ., Bull. 801. 7 pp.

§ 141-3 Wild bees important in alfalfa pollination - toxicity of residues on foliage.

(a) When required. (1) End-use products. Data on residual toxicity are required by 40 CFR § 158.155 to support the registration of each end-use product intended for foliar application to alfalfa grown for seed.

(2) Manufacturing-use products. Data on residual toxicity are required by 40 CFR § 158.155 to support the registration of each manufacturing-use product which can be legally and physically used to produce an end-use product subject to the data requirement in paragraph (a)(1) of this section.

(3) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this subdivision provides an additional discussion on this subject.

(b) Test standards. In addition to the general test standards contained in § 140-3, this study should meet the following standards:

(1) Substance to be tested. The test substance shall be a typical end-use product.

(i) If an applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product, the test substance shall be a product representative of a major formulation category which includes that end-use product. If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study should be performed with a typical end-use product for each such category.

(2) Test crop. Alfalfa should be used as the test crop.

(3) Species. Testing shall be performed on the alfalfa leafcutting bee, Megachile rotundata, and the alkali bee, Nomia melanderi.

(c) Reporting of data. Information to be reported should meet the general reporting guidelines of § 140-4 and the guidelines of § 141-2(c).

(d) Acceptable protocol. The following is an example of an acceptable protocol:

> The purpose of this test is to measure the toxicity of field-weathered pesticide residues to alfalfa leafcutting bees and alkali bees, as these species are important in the pollination of seed alfalfa. The methodology to be used is the same as that used for testing residual toxicity to honey bees (§ 141-2 of this subdivision) with the following exceptions:

(1) Alfalfa leafcutting bee: use 20-40 bees per cage;

(2) Alkali bee: use 15-30 bees per cage;

(3) Bees should be held at 85-88°F (29-31°C) during the test period.

(e) References. Additional information may be found in the following references:

Johansen, C., and J. Eves. 1967. Toxicity of insecticides to the alkali bee and the alfalfa leafcutting bee. Wash. Agric. Exp. Sta., Coll. of Agric., Wash. St. Univ. Circ. 475. 15 pp.

Johansen, C., D. Mayer, R. Madsen, and J. Curtis. 1974. Bee research investigations, 1974. Dept. of Entomol., Wash. St. Univ. Unpubl. 23 pp.

§ 141-4 Honey bee subacute feeding study. (Reserved)

§ 141-5 Field testing for pollinators.

(a) When required. (1) End-use products. Field testing of a pesticide for possible adverse effects on pollinators (honey bee, alfalfa leafcutting bee, and/or alkali bee) may be required by 40 CFR § 158.155 under the following conditions:

(i) Data from the honey bee subacute feeding study (§ 141-4) indicate adverse effects on colonies, especially effects other than acute mortality (reproductive, behavioral, etc.);

(ii) Data from residual toxicity studies (§§ 141-2 and 141-3) indicate extended residual toxicity; or

(iii) Data derived from studies with organisms other than bees indicate properties of the pesticide beyond acute toxicity such as the ability to cause reproductive or chronic effects.

(2) Manufacturing-use products. Data on field testing for pollinators are required by 40 CFR § 158.155 to support the registration of each manufacturing-use product which can legally and physically be used to produce a formulated product subject to the data requirement in paragraph (a)(1) of this section.

(3) See 40 CFR § 158.50, "Formulators' exemption," to determine whether these data must be submitted. Section II-A of this subdivision provides an additional discussion on this subject.

(b) Test standards. In addition to the general test standards contained in § 140-3, this study should meet the following standards:

(1) Substance to be tested. One end-use product, representative of each different type of formulation of the end-use products subject to paragraph (a)(1) of this section, shall be tested.

(2) Other standards. The standards for conducting these tests and the information which should be reported will be established by the Agency on a case-by-case basis following consultation between the applicant and the Agency.

(c) Reporting of data. Information to be reported should meet the general reporting requirements of § 140-4.

(d) References. Evaluation of pesticide hazard to bees through field testing has been accomplished using a wide variety of methods. Any field testing conducted to satisfy this requirement (§ 141-5 of this subdivision) should be preceded by consultation with the Agency.

Some acceptable protocols may be developed from the following references:

Atkins, E.L., Jr., L.D. Anderson, D. Kellum, and K.W. Neuman. 1976. Protecting honey bees from pesticides. Univ. of Calif., Div. of Agric. Sciences, Leaflet 2883. 14 pp.

Robinson, W.S., and C.A. Johansen. 1978. Effects of control chemicals for douglas-fir tussock moth Orgyia pseudotsugata (McDonnough) on forest pollination (Lepidoptera: Lymantriidae). Wash. St. Ent. Soc. "Melanderia" 30:9-56.

Series 142: NONTARGET INSECT TESTING - AQUATIC INSECTS

§ 142-1 Acute toxicity to aquatic insects. (reserved)

§ 142-2 Aquatic insect life-cycle study. (reserved)

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